REMARKS

In view of the above amendments and the following remarks, reconsideration of this application is respectfully requested.

Status of the Claims

Upon entry of the amendments presented herein, claims 10 and 21 will be pending. Claim 10 is hereby amended. New claim 21 is hereby added. Claims 1-9, 11-13, and 17-20 are hereby canceled without prejudice. Claims 14-16 were previously canceled without prejudice. As set forth in the remarks below, no new matter has been added by way of the amendments to the claims.

Newly added claim 21 is identical to currently amended claim 10, except that claim 21 further limits the "modification" to "methylation." Support for this amendment is found throughout the specification as originally filed. No new matter has been added by way of this amendment.

Request for Continued Examination

Applicants are submitting herewith a Request for Continued Examination (RCE), which submission is in full compliance with 37 C.F.R. § 1.114. The requisite RCE fee is also being submitted herewith.

Advisory Action

In response to applicants' previously filed ***Amendment & Response to Final Office Action*** (submitted May 11, 2010), the U.S. Patent and Trademark Office ("USPTO") has issued an ***Advisory Action*** (mailed May 21, 2010).

Under the Advisory Action, the USPTO stated that applicants' previously filed Amendment/Response was successful in overcoming all of the grounds for rejection, except for those discussed herein below. Applicants respectfully assert that the amendments to the claims and argument provided herein are sufficient to overcome the last remaining rejections.

Applicants respectfully request that they be given an opportunity to submit additional evidence, if the current claim amendments and remarks are not sufficient to overcome the currently pending final rejections. Therefore, applicants respectfully request that the Examiner not issue a final rejection in the next USPTO communication.

Indefiniteness Rejection – 35 U.S.C. § 112, second paragraph

Claim 10 remains rejected under 35 U.S.C. § 112, second paragraph, for alleged indefiniteness.

In view of the above claim amendments and the following remarks, and further in view of the remarks previously submitted by applicants in their Amendment/Response of May 11, 2010 (incorporated by reference herein), this rejection is respectfully traversed.

In the Advisory Action, the USPTO has indicated that it is unclear as to whether the method of claim 10 requires DNA fragments that include only a modified base, only a base, or both a modified base and a base.

The phrase "a mixture of DNA fragments having cohesive ends containing a base or a modified base" included in step (1)(a) of amended claim 10 is meant to cover a mixture containing at least one of the following:

(i) one or more DNA fragments having cohesive ends containing only a modified base(s);

(ii) one or more DNA fragments having cohesive ends containing only a base(s); and/or

(iii) one or more DNA fragments having cohesive ends containing both a modified base(s) and a base(s).

For example, when "an antibody specific to the modified base" is used in step (2) of amended claim 10, "a group of DNA fragments which form an immunocomplex with the antibody" includes the above-mentioned DNA fragments (i) and (iii), and "another group of DNA fragments which do not react with the antibody" includes the DNA fragments (ii). That is to say, DNA fragments (i) and (iii) can be separated from DNA fragments (ii).

By contrast, when "an antibody specific to the base that is not modified" is used in step (2) of amended claim 10, DNA fragments (ii) and (iii) can be separated from DNA fragments (i).

One of ordinary skill in the relevant art would readily understand the meaning of "a mixture of DNA fragments having cohesive ends containing a base or a modified base," as currently recited in amended claim 10.

In the Advisory Action, the USPTO also raised various other issues relating to the alleged indefiniteness of claim 10. Applicants have amended the claims to address each of the issues raised by the USPTO in the Advisory Action and Final Office Action. No new matter has been added by way of the amendments to claim 10.

In view of all of the foregoing, applicants respectfully submit that the rejection of claim 10 for indefiniteness is improper and should be withdrawn.

Obviousness Rejection – 35 U.S.C. § 103(a)

Claim 10 remains rejected under 35 U.S.C. § 103(a) for alleged obviousness over U.S. Patent No. 7,186,512 to Martienssen et al. (*Martienssen*) in view of U.S. Patent No. 7,247,428 to Makrigiorgos (*Makrigiorgos*).

In view of the above claim amendments and the following remarks, and further in view of the remarks previously submitted by applicants in their Amendment/Response of May 11, 2010 (incorporated by reference herein), this rejection is respectfully traversed.

Martienssen and *Makrigiorgos* were previously discussed by applicants in their Amendment/Response of May 11, 2010. Applicants continue to assert that the arguments presented therein are sufficient to overcome this rejection. However, in view of the recently issued Advisory Action, and in view of the further amendments to claim 10 (submitted herewith), applicants provide further arguments to overcome this rejection.

In the Advisory Action and in the telephonic interview with the Examiner, the USPTO indicated that more evidence may be required to support applicants' arguments that the type of restriction enzyme recited in amended claim 10 is functionally different from the

restriction enzymes described in *Martienssen* used to support the currently pending obviousness rejection.

The USPTO noted that it was basing this rejection in part on the restriction enzyme categories listed in the *New England BioLabs, Inc.* (NEB) reference cited in the Final Office Action (at page 10). The USPTO indicated that it would be helpful for applicants to provide further evidence (e.g., a journal article) to support the view that the restriction enzymes described in *Martienssen* and in the NEB reference are different from the one claimed in currently amended claim 10 of the present application.

Therefore, as further support for the view that the restriction enzymes ("methyl-dependent restriction enzymes" and "methyl-sensitive restriction enzymes") described in *Martienssen* are different from the one recited in the claims of the present application, enclosed please find:

(i) ***Reference A***: New England BioLabs (NEB), "Dam(G^mATC), Dcm(C^mCWGG) and CpG(^mCG) Methylation" (printed from the NEB website on July 27, 2010); and

(ii) ***Reference B***: McClellan et al., *Nucleic Acids Res.* 22(17):3640-3659 (1994).

Reference A is the same as that cited in the Final Office Action, except that the "CpG" column is missing from the reference attached to the Final Office Action. *Reference B* is described in *Martienssen* (column 9, lines 63-64).

As shown in *Reference A* and *Reference B*, the category of restriction enzymes covered by amended claim 10 are distinct from those included in *Martienssen* and in the NEB reference cited in the Final Office Action, as discussed below.

A. Restriction enzymes claimed in the present application

Examples of the "restriction enzyme which can digest a DNA regardless of the presence or absence of a modification in a recognition site" are concretely disclosed in the present invention. For example, BsaWI, BsoB1, BssSI, MspI, TaqI, XmaI, BsaJI, and PspAI are listed in **Table 1** of the present specification, as the restriction enzymes that can be used when the modification to be analyzed is "methylation of cytosine in higher animals."

BsaWI, BsoBI, BssSI, MspI, TaqI, and BsaJI are represented in the CpG column of *Reference A* by the green circle, which means "Not Sensitive." This indicates that these restriction enzymes can cleave their recognition sites in both the presence and absence of methylated cytosine in a CpG site (5'-CG-3' sequence in higher animals; *see* paragraph [0039] of the present specification).

PspAI is not described in *Reference A*, but has the same properties.

XmaI is represented by the blue rhombus, which means "Impaired." XmaI can cleave its recognition site in the presence of methylated cytosine in a CpG site, by lengthening the digestion time.

B. "Methyl-dependent restriction enzymes"

Martienssen discloses McrBC, McrA, MrrA, and DpnI (column 9, lines 60-61) as the "methyl-dependent restriction enzymes." Although McrBC, McrA, and MrrA are not disclosed in *Reference B*, DpnI is disclosed on page 3649.

DpnI cleaves DNA fragments when G in GATC is methylated (G^{m6}), but does not cleave them when G is unmethylated. This indicates that DpnI is a "methyl-dependent restriction enzyme."

C. "Methyl-sensitive restriction enzymes"

Martienssen discloses as the "methyl-sensitive restriction enzymes" PstI, BstNI, FseI, MspI, CfoI, and HpaII (column 9, line 62), all of which are disclosed in *Reference B*.

PstI, FseI, CfoI, and HpaII do not cleave DNA fragments containing a methylated base. This indicates that PstI, FseI, CfoI, and HpaII are typical "methyl-sensitive restriction enzymes."

MspI does not cleave DNA fragments containing ^{m5}CCGG or ^{hm5}C^{hm5}CGG, in which the outer C is methylated at the 5-position, and BstNI does not cleave DNA fragments containing specific sequences such as ^{hm5}C^{hm5}CWGG and C^{m4}CWGG. This also indicates that MspI and BstNI are "methyl-sensitive restriction enzymes."

D. MspI

MspI is a restriction enzyme that may be used in the present invention wherein the modification is methylation of cytosine in higher animals (*see* above Item A), and is also exemplified as the "methyl-sensitive restriction enzymes" disclosed in *Martienssen* (*see* above Item C).

The reason why MspI is defined as the methyl-sensitive restriction enzyme in *Martienssen* is that methylation of cytosine is not limited in *Martienssen* to methylation in higher animals. Methylations in the above sequences ^{m5}CCGG and ^{hm5}C^{hm5}CGG which are not cleaved by MspI are not observed in higher animals, but are observed in, for example, prokaryotes. In this connection, *Martienssen* discloses that "(t)he invention can be used over a broad range of organisms, including fungi, animals and plants" (column 15, lines 66-67), which apparently indicates that methylation in *Martienssen* is not limited to that in higher animals.

In view of all of the foregoing and further in view of applicants' previously submitted arguments in their Amendment/Response of May 11, 2010, applicants respectfully submit that this rejection is improper and should be withdrawn.

CONCLUSION

Claims 10 and 21 are now under consideration in this case. In view of the foregoing, applicants respectfully submit that the claims of the present application are in condition for allowance and such allowance is earnestly solicited.

If any unresolved issues remain that might prevent the prompt allowance of the present application, the Examiner is respectfully encouraged to contact the undersigned at the telephone number listed below to discuss these issues.

A two-month extension is now required. Therefore, to satisfy the two-month extension of time under 37 C.F.R. § 1.17(a)(2) (Large Entity), submitted herewith via EFS-Web is payment in the amount of **$490**.

Also submitted herewith via EFS-Web is payment in the amount of **$810** for the RCE fee under 37 C.F.R. § 1.17(e) (Large Entity).

The Commissioner is hereby authorized to charge any fees that may have been overlooked, or to credit any overpayments of fees, to Deposit Account No. 08-1935.

Respectfully submitted,

HESLIN ROTHENBERG FARLEY & MESITI P.C.

By: **/Andrew K. Gonsalves/**

Dated: **August 11, 2010**

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